

Ex vivo OT-1 cross-priming assay and secreted cytokine measurement

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An abbreviated version of this protocol was published in Science Immunology in Jun 2022

ATR-mediated CD47 and PD-L1 up-regulation restricts radiotherapy-induced immune priming and abscopal responses in colorectal cancer

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Detailed protocol

Dear Daniela,

Thanks so much for your interest in the ex vivo OT-1 cross-priming assay. In this experiment, we isolated the CD11b⁺CD11c⁺ DCs (mostly cDC1), CD11b⁺CD11c⁺ DCs, or CD11b⁺CD11c⁺ F4/80⁺ TAMs from the irradiated MC38-OVA tumors by cell sorting (BD FACS Aria III/Jazz) 5 days after 8 Gy or sham irradiation ± anti-SIRPα and anti-PD-1 (fig. S19). Splenic OT-1 cells were harvested from 6- to 12-week-old C57BL/6-Tg(TcrαTcrβ)1100Mjb/J mice (#003831) using the CD8α⁺ T Cell Isolation Kit. The unstimulated OT-1 cells were cocultured with different APC subsets (OT-1:APC = 140,000:70,000) or anti-SIRPα (20 μg/ml) and/or anti-PD-1 (12.5 μg/ml) antibodies alone in RPMI 1640 with 10% FBS and 1% penicillin-streptomycin plus 50 μM β-mercaptoethanol for 48 hours in a 96-well plate. Cells were treated with GolgiPlug 6 hours before intracellular cytokine staining, and the levels of PD-1, CD44, ICOS, Granzyme B, Ki-67, and IFN-γ in OT1 cells were analyzed using flow cytometry. Secreted cytokines in the supernatant were measured using the CBA Mouse Th1/Th2/Th17 Cytokine Kit (BD Biosciences #560485). The protocol of the cytometric bead array can be found on the BD website (<https://www.bdbiosciences.com/en-au/products/reagents/immunoassay-reagents/cba/cba-kits/mouse-th1-th2-th17-cba-kit.560485>).

Best regards,
Rodney

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

- Hsieh, C. and Curran, M. (2022). Ex vivo OT-1 cross-priming assay and secreted cytokine measurement. Bio-protocol Preprint. bio-protocol.org/prep1779.
- Hsieh, R. C., Krishnan, S., Wu, R., Boda, A. R., Liu, A., Winkler, M., Hsu, W., Lin, S. H., Hung, M., Chan, L., Bhanu, K. R., Srinivasamani, A., De Azevedo, R. A., Chou, Y., DePinho, R. A., Gubin, M., Vilar, E., Chen, C. H., Slay, R., Jayaprakash, P., Hegde, S. M., Hartley, G., Lea, S. T., Prasad, R., Morrow, B., Couillault, C. A., Steiner, M., Wang, C., Venkatesulu, B. P., Taniguchi, C., Kim, Y. S. B., Chen, J., Rudqvist, N. and Curran, M. A. (2022). ATR-mediated CD47 and PD-L1 up-regulation restricts radiotherapy-induced immune priming and abscopal responses in colorectal cancer. Science Immunology 7(72). DOI: [10.1126/sciimmunol.abl9330](https://doi.org/10.1126/sciimmunol.abl9330)

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